Veterinary Parasitology 193 (2013) 134–140

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Comparison between the action of nematode predatory fungi *Duddingtonia flagrans* and *Monacrosporium thaumasium* in the biological control of bovine gastrointestinal nematodiasis in tropical southeastern Brazil

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ARTICLE INFO

Article history:
Received 27 March 2012
Received in revised form
19 November 2012
Accepted 10 December 2012

Keywords:
Cattle-Nematoda
Biological control
Duddingtonia flagrans
Monacrosporium thaumasium
Bovine

ABSTRACT

Sodium alginate pellets of the nematode predatory fungi *Duddingtonia flagrans* and *Monacrosporium thaumasium* were evaluated in the biological control of bovine gastrointestinal nematodiasis. Three groups (A–C) of ten six month old male Nelore bulls were kept in paddocks of *Brachiaria decumbens* for 12 months. Each animal of group A received 1 g/10 kg of body weight (b.w.) of pellets of *D. flagrans* (0.2 g of fungus/10 kg b.w.) and of group B, 1 g/10 kg of b.w. of pellets of *M. thaumasium* (0.2 g of fungus/10 kg b.w.), twice a week, for 12 months. Animals of the group control received no fungus. The monthly averages of egg count per gram of feces of the animals of groups A and B were 56.67% and 47.8% smaller, than the animals of group C ($p < 0.05$), respectively. Treatment of bulls with pellets containing the nematophagous fungi *D. flagrans* and *M. thaumasium* can be used as an alternative treatment of bovine gastrointestinal nematodiasis, however, *D. flagrans* was more efficient than *M. thaumasium* for the biological control in the environmental conditions of the present study.

1. Introduction

Research on the application of the nematode-trapping fungi *Duddingtonia flagrans* (Larsen et al., 1995; Sarkunas et al., 2000; Dias et al., 2007) and *Monacrosporium thaumasium* (Alves et al., 2004; Araújo et al., 2004) for treating bovine gastrointestinal nematodiasis has demonstrated their potential as biological control agents against the free-living stages of parasitic nematodes in livestock under experimental and natural conditions. However, most studies of biological control in cattle have been conducted in temperate regions (Larsen et al., 1995; Sarkunas et al., 2000) with dairy cattle that is usually kept in pasture only in the grazing season. There are no reports of previous studies evaluating biological control with *D. flagrans* or *M. thaumasium* in extensive systems of beef cattle and, due to weather conditions, maintained on pasture throughout the year.

Sodium alginate based formulations have been evaluated experimentally in the control of animal parasitic nematodes by some research groups. Such formulations have provided good results under laboratory and field

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0304-4017/$ – see front matter.
http://dx.doi.org/10.1016/j.vetpar.2012.12.005

conditions (Araújo et al., 2000). Various nematophagous fungi have been successfully used in formulations based on sodium alginate against gastrointestinal helminthes; however, few studies have been conducted with the fungi *M. thaumasium* (Alves et al., 2004; Silva et al., 2009) or *D. flagrans* (Campos et al., 2007; Silva et al., 2009) using these formulations and under natural conditions.

The objective of this study was to evaluate the effectiveness of the nematophagous fungi *D. flagrans* and *M. thaumasium* in sodium alginate pellets in the biological control of gastrointestinal nematodiasis in beef cattle raised in the field, in a tropical climate.

2. Materials and methods

2.1. Area of study

The experiment was carried out in the experimental farm of the Federal University of Viçosa, located in the municipality of Florestal, state of Minas Gerais, southeast region of Brazil, 19°53′22″south latitude and 44°25′57″ west longitude, from May 2010 to June 2011.

The paddock's topography is flat to hilly (29% flat, 54% undulating and 17% hilly), with an average altitude of 750 m and native vegetation of forest-cerrado transition zone. The climate is tropical with a dry season (Rating Köppen-Geiger climate: Aw), with annual average maximum temperature of 28 °C and minimum of 13.9 °C.

2.2. Fungal cultures

Two isolates of the predatory fungi *D. flagrans* (AC001) and *M. thaumasium* (NF34a) were kept in test tubes containing corn meal agar 2% (2% CMA, Difco®, USA), at 4 °C in the dark. These isolates came from a Brazilian soil and belonged to the mycology collection of the Federal University of Viçosa, Brazil. To induce the formation of the fungal mycelium, culture discs of 5 mm in diameter were transferred to 250 mL Erlenmeyer flasks containing 150 mL of potato dextrose (Difco®, USA) liquid medium, pH 6.5, under the agitation of 120 rpm, in the dark, 26 °C, for 10 days. After this period, the mycelia were harvested with a platinum loop, and weighed in an analytic scale for the future production of the pellets, which were made in a sodium alginate matrix, according to Walker and Connick (1983) and modified by Lackey et al. (1993).

2.3. Experimental animals

In the beginning of the experiment, thirty 6-month-old male Nelore bulls, with average body weight of 120 kg were previously treated with 10% albendazole (Mogivet Lab®, Brazil), at an oral dose of 7.5 ml/10 kg of b.w. Fifteen days after the antihelminthic treatment, the animals were separated into three groups (A–C) of 10 bulls each, based on the average weight. The bulls were allocated to three 15.0 ha paddocks of *Brachiaria decumbens*, naturally infested with gastrointestinal parasite helminths, due to the previous grazing by young and adult animals. Each group was allocated to only one paddock without rotational grazing between the groups during the experimental period. Each animal of group A received 1 g of pellets (0.2 g of fungal mycelium) for each 10 kg of b.w. containing the fungus *D. flagrans* (AC 001) while, in group B, each animal received 1 g of pellets (0.2 g of fungal mycelium) for each 10 kg of b.w. containing the fungus *M. thaumasium* (NF34a). The animals of group C received 1 g of fungus-free pellets for each 10 kg of b.w. All the animals received the pellets orally, twice a week, mixed in concentrated and balanced ration provided for beef cattle (13% of total protein – Federal University of Viçosa), and water *ad libitum* during 12 months, starting from May 2010.

After the introduction of the bulls into the paddocks, samples of feces were collected, once a week, directly from the rectum, to determine the number of nematode eggs per gram of feces (EPG), according to Gordon and Whitlock (1939). Every day, meteorological data were recorded in a specialized station in the region, referring to the averages of the maximum, average, and minimum monthly temperatures and rainfall.

Fecal samples were collected from the animals to observe the growth of the fungi, once a week, two days after treatment with fungi. The feces were incubated in plates containing water agar 2% and 100 L3 recovered from the coprocultures and put into a drying oven at 25 °C, for 10 days to confirm the passage and the predatory ability of the fungi through the gastrointestinal tract of cattle and growth in the feces. Simultaneously to the EPG exam, coprocultures were carried out, for each animal, according to the methodology described by Roberts et al. (1952). The identification of the infectant larvae in the coprocultures was performed according to Keith (1953). Egg count per gram of feces (EPG) and larvae recovered from coprocultures of animals from both treated and control groups were recorded, and percentage of larval reduction was determined according to Mendoza-De-Guives et al. (1999): reduction (%) = mean L3 recovered from control group − mean L3 recovered from treated group × 100/mean L3 recovered from control group.

Every 15 days, herbage samples were collected in each paddock of groups, in a zigzag pattern from alternated points, 20 cm and 20–40 cm far from the fecal pats, according to Amarante et al. (1996). Then, a 500 g herbage sample was weighed, and parasitic nematode larvae were recovered following the procedure of Lima (1989). The samples were incubated in a drying oven at 100 °C for 3 days to determine the dry matter content. Data were transformed into larvae per kg of dry matter.

The EPG, number of infective larvae (L3) recovered from the feces and paddocks, and correlation between EPG and recovered L3 were compared over the experimental period. The weight of the animals was compared during the months of the experiment, starting from June 2010. EPG data were transformed into $\log(x+1)$ prior to analysis. Data was examined by analyses of variance and Tukey's multiple comparison test with 1% probability. Correlation analyses were performed by Pearson's correlation test ($p < 0.001$).

3. Results

The monthly average values of EPG counts are shown in Fig. 1. In the first three months of the experiment (May,

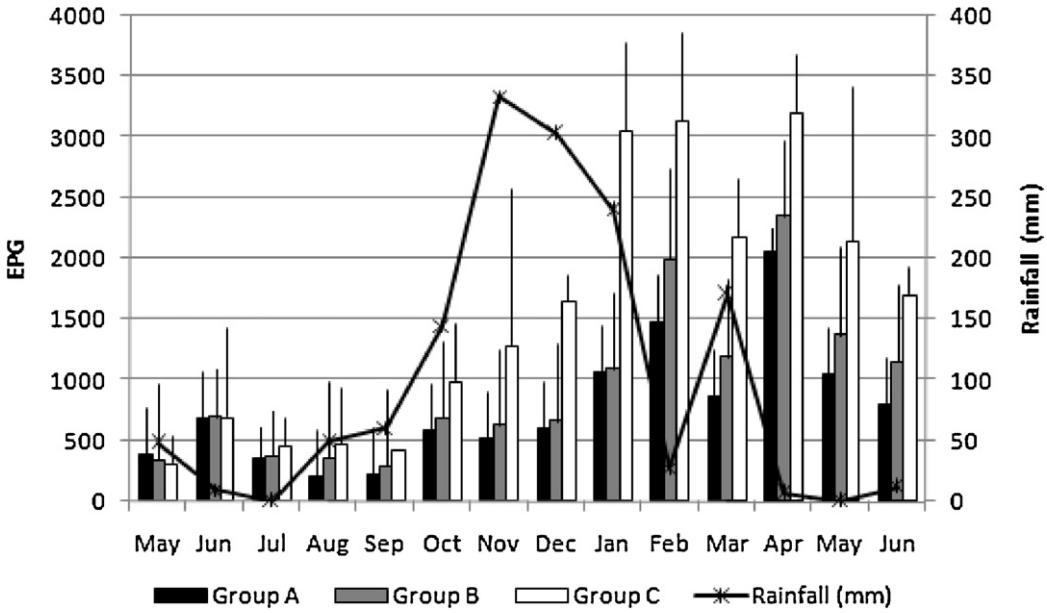

Fig. 1. Monthly averages and standard deviations of the countings of eggs per gram of feces (EPG) of the animals in the groups treated with the nematophagous fungi *D. flagrans* and *M. thaumasium* (1 g of fungus/10 kg of body weight) and the control group, collected from May 2010 to June 2011, Florestal, Minas Gerais, Brazil.

June and July 2010) no statistical difference was observed ($p > 0.05$) between the groups treated with fungi (A and B) and the control group (C). In the first month of the study (May 2010), the low EPG number was probably due to the previous anthelmintic treatment. EPG values increased during the rainy season (October from March) and peaked in April 2011 (2060, 2400, 3200 eggs in groups A, B and C, respectively) at the end of the rainy season (Fig. 1).

The EPG counts of animals treated with *D. flagrans* (group A) and *M. thaumasium* (group B) were significantly lower than those of the control group from August 2010 to June 2011 ($p > 0.05$). However, the EPG counts of animals treated with *D. flagrans* were significantly lower than those of the animals treated with *M. thaumasium* on August 2010 and February, March, April, May, June 2011. There was no difference between the EPG of the two groups treated with fungus in September, October, November, December 2010 and January 2011.

The monthly average of the EPG of the animals in the group treated with pellets containing the fungus *D. flagrans* was 56.7% lower than that of the animals in the control group at the end of the experiment. For the animals of group B treated with pellets containing the fungus *M. thaumasium*, the reduction observed was of 47.8% in comparison with the animals in the control group. EPG decreased significantly more in *D. flagrans* than in *M. thaumasium* at the end of the experiment.

Fig. 2 shows the maximum, average and minimum temperature and monthly rainfall.

Table 1 shows the percentage values corresponding to the infectant larvae (L3) recovered from the coprocultures.

The coprocultures showed that *Cooperia* sp. was the most prevailing gastrointestinal parasitic nematode in the experiment in all the groups, with percentages of 48.4%, 53.1%, and 44.1% for the animals of the groups A, B, and C, respectively. *Haemonchus* was 38.2, 34.7 and 43.4% and *Oesophagostomum* was 12.1, 10.9 and 10.4%. Other species found in smaller quantities in the groups A, B and C were *Bunostomum* sp. (0.8, 0.6 and 1.2%) and *Strongyloides* sp. (0.5, 0.7 and 0.9%), respectively. No difference ($p > 0.01$) was found in the proportion of different genera between the animals of the three groups.

Table 2 shows the absolute values of L3 per kg of dry matter obtained from pastures grazed by the three groups of cattle.

The larval numbers of *Cooperia*, *Haemonchus* and *Oesophagostomum* of the group treated with *D. flagrans* recovered from the pasture (per kg dry matter) reduced by 60.5, 53.2 and 47.5%. In the group treated with *M. thaumasium* the reduction was 33.6, 26.6 and 57.5% respectively ($p < 0.01$). *M. thaumasium* provided a lower percentage reduction of *Cooperia* and *Haemonchus* in the pasture in relation to *D. flagrans*.

In both groups treated, the number of L3 recovered up to 20 cm differed from that found 20 and 40 cm from the fecal pat. Eighty-four percent of the total L3 recovered from the paddocks were in the distances of up to 20 cm from the fecal pat and only 16% was recovered within 20–40 cm from the fecal pats in the group treated with *D. flagrans*.

The same pattern was found in the group treated with *M. thaumasium*, where 91% of the larvae were up to 20 cm from the fecal pat and only 9% from 20 to 40 cm. In the control group the highest percentage of L3 was also up to 20 cm from the fecal pat (73%), but there was a greater amount of L3 recovered from 20 to 40 cm (27%) compared with the treated groups.

The percentage reduction of L3 in relation to the group control in the distances of up to 20 and 20–40 cm from

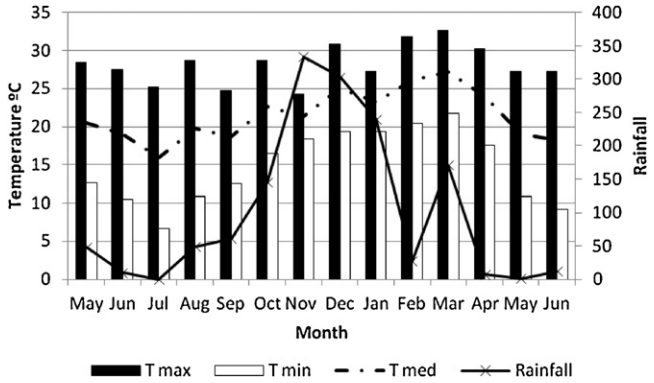

Fig. 2. Averages of maximum, average and minimum monthly temperatures (°C) and monthly rainfall (mm^3) recorded from May 2010 to June 2011, Florestal, MG, Brazil.

Table 1
Percentage values corresponding to the infectant larvae (L3) recovered from the coprocultures of the groups treated with the nematophagous fungi *D. flagrans* (Group A) and *M. thaumasium* (group B) (1 g of fungus/10 kg of body weight) and control group (group C), collected from May 2010 to June 2011, in Florestal, Minas Gerais, Brazil.

	Group A			Group B			Group C		
	Coop	Haem	Oeso	Coop	Haem	Oeso	Coop	Haem	Oeso
May	45	55	0	39	61	0	61	39	0
Jun	29	71	0	21	71	4	75	25	0
Jul	62	35	3	72	24	0	41	53	6
Aug	68	27	5	74	18	3	29	63	8
Sep	75	11	14	82	10	2	22	68	10
Oct	65	25	10	75	8	17	22	60	18
Nov	76	11	9	77	9	14	17	56	7
Dec	36	8	56	61	29	8	18	52	25
Jan	55	33	12	41	10	49	40	41	19
Feb	44	25	31	61	27	14	35	29	32
Mar	50	34	6	76	5	20	46	49	5
Apr	32	54	14	14	77	9	69	24	7
May	26	64	10	28	67	5	63	28	9
Jun	15	82	0	23	70	7	79	21	0
Average	48.4	38.2	12.1	53.1	34.7	10.9	44.1	43.4	10.4
S.D.	18.5	22.5	14.4	23.6	26.8	12.2	21.0	15.4	9.3

Table 2
Values of L3 per kg of dry matter obtained from pastures grazed by the groups treated with the nematophagous fungi *D. flagrans* (group A) and *M. thaumasium* (group B) and control group (group C).

	Group A			Group B			Group C		
	Coop	Haem	Oeso	Coop	Haem	Oeso	Coop	Haem	Oeso
May	45	55	0	39	61	0	61	39	0
Jun	29	71	0	21	71	4	75	25	0
Jul	62	35	3	72	24	0	41	53	6
Aug	68	27	5	74	18	3	29	63	8
Sep	75	11	14	82	10	2	22	68	10
Oct	65	25	10	75	8	17	22	60	18
Nov	76	11	9	77	9	14	17	56	7
Dec	36	8	56	61	29	8	18	52	25
Jan	55	33	12	41	10	49	40	41	19
Feb	44	25	31	61	27	14	35	29	32
Mar	50	34	6	76	5	20	46	49	5
Apr	32	54	14	14	77	9	69	24	7
May	26	64	10	28	67	5	63	28	9
Jun	15	82	0	23	70	7	79	21	0
Average	48.4	38.2	12.1	53.1	34.7	10.9	44.1	43.4	10.4
S.D.	18.5	22.5	14.4	23.6	26.8	12.2	21.0	15.4	9.3

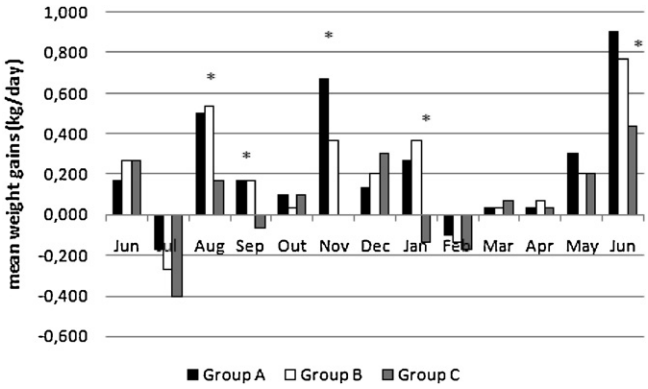

Fig. 3. Mean weight gains (kg/day) of the groups treated with *D. flagrans* and *M. thaumasium* and control from May 2010 to June 2011, Florestal, MG, Brazil. Significant difference ($p < 0.01$) between the treated group and the control denoted by asterisk – Tukey test.

the fecal pat was 64.5% and 73%, respectively, for group A, group B showed percentage reductions of 47.3% and 58%, in the same distances. *D. flagrans* showed larger percentage reduction of L3 in the pasture compared with *M. thaumasium* ($p > 0.01$), but no difference was observed between the groups A and B in October, November and December 2010 and January 2011.

The correlation coefficient between mean EPG of each group and infective larvae recovered from the paddocks of group A within the distance from 0 to 20 cm from fecal pats was 0.74 and for the distance between 20 and 40 cm was 0.65. For group B, the correlation coefficient between EPG and infective larvae recovered within 0–20 cm from the fecal pats was 0.67 and between 20 and 40 cm was 0.62. For group C, the correlation coefficient recovered within 0–20 cm from the fecal pats was −0.68 and within 20–40 cm was −0.54. For the treated groups (A and B) there was a strong positive correlation between EPG and infective larvae, although in for group C a negative correlations between EPG and infective larvae was observed.

Fig. 3 shows the mean weight gains of animals of the three groups. The weight gain of the animals of the treated groups differed from those of the control group.

Fig. 4 shows scanning electron micrographs of fungi *D. flagrans* (4A) and *M. thaumasium* (4B) recovered from the fecal samples of the treated animals. The analysis of the plates showed the fungal growth and the ability to predate L3 of *D. flagrans* and *M. thaumasium*. Presence of nematophagous fungi was not detected in the feces of the control group during the experiment.

3.1. Discussion

In the present work, the animals in the group treated with the fungus *D. flagrans* had a reduction in EPG of 56.67%, compared with the animals in the control group. Several works using the fungus *D. flagrans* in ruminants also recorded smaller monthly average values of EPG counts of treated animals in relation to the control group (Dimander et al., 2003; Fontenot et al., 2003; Araújo et al., 2004; Silva et al., 2009). Dias et al. (2007) in a study with the same *D. flagrans* isolate obtained 31% reduction in EPG of treated crossbred Holstein-Zebu cattle.

The group treated with *M. thaumasium* had an EPG reduction of 47.8% in relation to the control group. Studies using the fungus *M. thaumasium* in ruminants recorded monthly average values of EPG counts lower in treated animals. Alves et al. (2004), with crossbred Holstein-Zebu

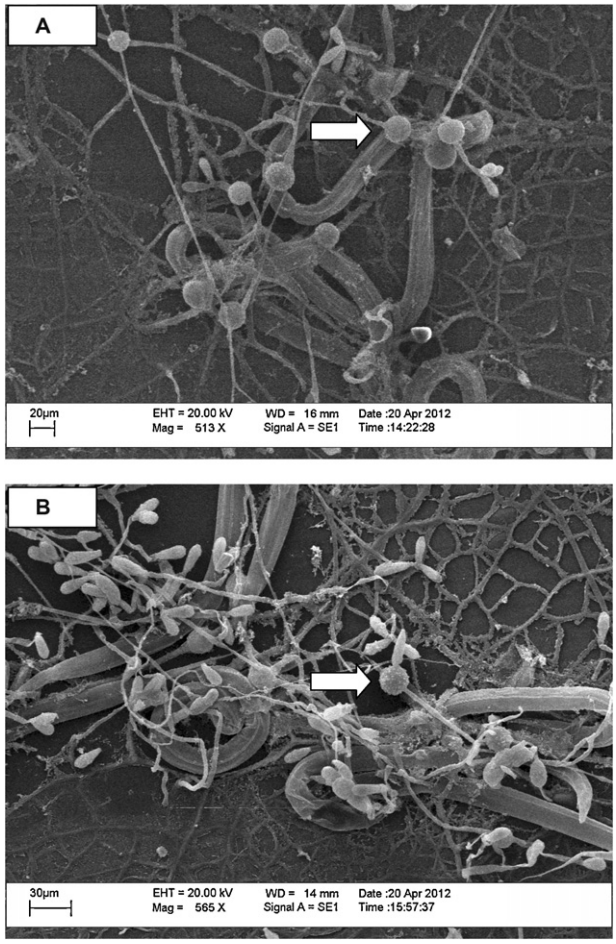

Fig. 4. Scanning electron micrographs of trap formation process and interaction of the fungus *D. flagrans* (Fig. 4A) and *M. thaumasium* (Fig. 4B) with trichostrongyles larvae on dialysis membrane surface.

cattle treated with same isolate of *M. thaumasium* obtained 88.8% reduction in EPG after four months of trial.

There are not previous records in the literature of studies involving nematophagous fungi in biological control in cattle during the whole year, going through the dry and rainy seasons in a tropical climate. There are no reports of studies comparing two fungi *in vivo* simultaneously in the same environmental conditions. In this study *D. flagrans* and *M. thaumasium* were proven effective in the reduction of EPG counts in cattle, however, it showed that in these climatic conditions, *D. flagrans* was more effective than *M. thaumasium* in reducing EPG. It was also observed that the isolate of *M. thaumasium* was less effective in the months with low rainfall (April, May and June 2011). The production of chlamydospores may be related with these results, because it is knew that *D. flagrans* produces more chlamydospores than *M. thaumasium* (Fig. 4).

Araújo et al. (1998) demonstrated that in the southeast region of Brazil, *Cooperia* spp. and *Haemonchus* spp. were the most prevailing gastrointestinal nematodes in bovine followed by *Oesophagostomum* spp. These results are also in accordance with the present work.

Araújo et al. (2007) recorded a larva decrease in the coprocultures of the animals treated with the fungus *M. thaumasium* in the Brazilian semiarid. Larsen et al. (1998) evaluated the potential of the fungus *D. flagrans* in the control of the free living stages of the animal parasites and achieved a reduction of more than 80% in the number of L3 in paddocks. In present work, the effectiveness of the fungi *D. flagrans* and *M. thaumasium* against L3s of *Cooperia*, *Haemonchus* and *Oesophagostomum* was observed in the treated groups. In relation to differential percentage of infective larvae *Cooperia* was the most prevalent genus in all groups. In Brazil, Furlong et al. (1985), Alves et al. (2004) and Araújo et al. (2004) also reported predominance of the genus *Cooperia* in the pasture in comparison with other genera.

Climatic conditions, such as temperature and rainfall favored the development of free-living stages and migration to the herbage (Quinelato et al., 2008). The number of L3 recovered from the paddocks in the distances up to 20 cm and 20–40 cm from the fecal pat was similar ($p > 0.05$) for the animals in groups A and B in the months with highest rainfall rates. In these months, a larger number of trichostrongyles larvae were recorded in pasture. The lowest rainfall rates were observed in May, June and July 2010 with 48.2, 9.8 and 0 mm^3 in April, May and June 2011 with 7.4, 0.8 and 12 mm^3 respectively, coinciding with a low count of trichostrongyle larvae recovered from the pastures. Once the larvae migrate from feces onto pastures, it is possible that the migration 20 cm beyond the fecal pat is performed by few larvae.

In relation to weight gain, all groups showed continued growth in weight during the rainy season and lost of weight during the dry season, relationed with a marked reduction in production and nutritive value of the pasture. Even though, difference was observed in the weight gain of animals of the treated groups in comparison with the animals of the control group. This reinforces the fact that the administration of pellets with fungi was favored by the treatment of the animals, with reduction of infective larvae in the pasture where the animals of this group were likely to be contaminated by a lower infective larval burden. A higher weight gain in the animals in relation to the control group was also observed by Araújo et al. (2007) when testing the fungus *M. thaumasium* in goats in the Brazilian semiarid. Braga et al. (2009) in a field study with horses using the nematophagous fungus *D. flagrans* observed significant differences in weight gain among the groups treated with the fungus and the control. In the period from March to June the rate of weight increase does not look much different in the 2 groups. There was significant difference ($p > 0.01$) for weight gain between the two treated groups only in three months of the study (July, October and November 2010), when the animals treated with *D. flagrans* showed higher mean weight. Regarding the linear coefficients of regression presented in this work, the results showed that there is a positive correlation between the reduction of EPG of the animals and the reduction of L3 in the pastures of the treated group. Dias et al. (2007) reported that there may be dependence between EPG and infective larvae recovered from pasture. In this study, the significant correlations between EPG and L3 in pasture indicate that environmental contamination was an important factor in increasing the parasite load of animals.

3.2. Conclusion

The treatment of beef cattle with alginate pellets containing the nematophagous fungi *D. flagrans* and *M. thaumasium* can be used as an alternative method in the control of bovine gastrointestinal nematodes. The fungus *D. flagrans* was shown more promising than *M. thaumasium* for continuous use during the dry and rainy seasons in tropical regions of low rainfall.

Acknowledgments

This study was supported by the Research Project of the Federal University of Viçosa, Brazil. Thanks are also due to FAPEMIG for the financial support and CAPES for the scholarships granted.

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